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Biological Activity of Bacteriophage Ghosts and "Take-Over" of Host Functions by Bacteriophage

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INTRODUCTION

Empty protein coats, or ghosts, were observed very early in the study of the T series of bacterial viruses. In 1946, Hook et al. (61) observed that some preparations of these phage contained "images of characteristic outline but seemingly empty" heads. These "forms or ghosts" were considered to be "a most interesting and probably significant finding." In 1950, Anderson (4) discovered that rapid dilution of a suspension of T-even phage from a concentrated salt solution caused inactivation of their plaque-forming ability with the concomitant appearance in the electron microscope of phage with empty head membranes. In 1951, Herriott showed that although these osmotically shocked phage, or ghosts, had lost their infectivity, they retained their capacity to kill the host cell (50). It was observed later that ghosts have the ability to inhibit a wide variety of host functions (13, 29, 30, 40, 50–52, 54, 75, 76) as, of course, do viable phage (*see, for instance*, 5, 24, 119), but it is not known whether they do so by mechanisms which are at all related. To answer this question, we have been studying some aspects of the metabolism of the ghost-infected cell to try to determine whether the cell death that occurs following the attachment of a phage ghost to a susceptible cell is an integral part of the ability of the phage to shut down host macromolecular syntheses or a side effect of cell surface damage which in the course of normal infection would be repaired by phage-induced enzymes. We hope that with a clearer understanding of how cell replication may be so effectively interrupted some light will be shed on the problem of the control of normal cell replica-

tion. Therefore, this paper is devoted to a review of those activities of phage and ghosts which may relate to the ability of the phage to "take-over" the synthetic machinery of the host to disrupt cell replication.

METHODS UNIQUE TO THE STUDY OF GHOSTS

Preparation of Ghosts

Anderson's (4) original preparations of ghosts were made by incubation of phage in 4 M sodium chloride followed by rapid dilution with water. He found that if the solute concentration was lowered slowly by, for instance, dialysis, no virus inactivation or head disruption occurred. Of the phages he tested, T2, T4, and T6 were shown to be susceptible to the shocking procedure, whereas T1, T3, and T7 were resistant. Sodium chloride, sodium sulfate, ammonium acetate, ammonium sulfate, ethylene glycol, glycine, glycerol, glucose, and sucrose have since been used to prepare ghosts of the T-even phages (4, 13, 29, 30, 40, 50, 51, 77); use of magnesium sulfate does not always result in the production of ghosts, however (51, 77). Rapid 10- to 100-fold dilution of phage equilibrated with concentrated solutions of these agents results in 98 to 99.6% inactivation of their plaque-forming ability, apparently by rupturing the phage head and causing the deoxyribonucleic acid (DNA) to be extruded into the medium. Some mutants which are osmotic-shock resistant map in the head protein gene (16, 77) and appear to have head membranes which are more permeable to solute molecules than are wild-type phage (77). This fact supports the conclusion that inactivation results from changes in the phage head, but it is not known whether the head is the only phage constituent damaged during the shocking procedure.

Leibo and Mazur (77) performed a thorough study of the survival of T4 phage after osmotic shock and found that very minor differences in procedure have marked effects on phage survival, with dilution rate and final salt concentration being most responsible for the variation. They show that when T4 phages are rapidly diluted from 3 M NaCl, they are subject to two separate effects—those due to a large rapid change in osmotic pressure (osmotic shock) and those due to exposure to low (0.03 to 0.00003 M) salt concentrations. $T4BO_1$, which is relatively resistant to osmotic shock when diluted some 100-fold from concentrated salt solutions, is inactivated by exposure to very low salt concentrations. [It should be noted, though, that some (30 to 50%) inactivation of $T4BO_1$ does occur by dilution from 3 to 0.03 molal NaCl.] Although this second type of inactivation (i.e., that observed in low salt concentrations) is attributed to changes in the phage DNA, there is no rigorous proof of this. It is conceivable, therefore, that some other phage structure is being affected. It is frequently observed that when phage are osmotically shocked, there is a loss of particles which are able to inactivate bacteria (13, 28, 30, 54). If there were two mechanisms of inactivation operating under conditions normally used to produce ghosts, this phenomenon might be explained. This is further discussed in the section on the assay of ghosts.

Another interesting observation which Leibo and Mazur make is that nearly half of the osmotically shocked phage remain associated with their DNA (77). Prater also observed this (98). If this DNA were functionally bound to the ghost, it could account for the wide variation in properties of ghosts which have been reported (40, 51, 52, 76). To test this possibility, ^{14}C-thymidine-labeled T4 phage were subjected to osmotic shock by rapid 100-fold dilution from 2 M sodium acetate. It was found that, although more than 99% of the infectivity was lost, some 20% of the DNA, as measured by its thymidine label, remained associated with the ghosts through two cycles of differential centrifugation. If, however, the ghosts were treated with deoxyribonuclease after shocking, less than 1% of the label remained associated with the killing ability of the ghost. This residual label can be accounted for by remaining viable phage. It was further observed that the deoxyribonuclease treatment caused no changes in the ability of the ghosts to inhibit induced enzyme synthesis (D. Duckworth, *unpublished data*).

This confirms the observations of others who have seen that deoxyribonuclease treatment does not affect the biological activities of ghosts. Bonifas and Kellenberger (13) and Hershey (54) found that the killing titer of ghost preparations is not affected by treatment with deoxyribonuclease, and Herriott and Barlow (51) showed that the lytic properties of ghosts are not changed by deoxyribonuclease treatment.

Assay of Ghosts

One of the major difficulties in interpreting the earlier work on phage ghosts stems from the lack of a reliable assay for ghosts. When a population of cells is infected with virus, the number of infected cells is statistically related to the number of particles added, according to the Poisson distribution (2). The distribution is such that a twofold difference in input multiplicity can cause a 10-fold difference in the number of uninfected

cells. The need for a quantitative assay, therefore, is not a trivial matter when one wishes to measure the effect of virus particles on a culture of cells. Several of the earliest investigators who worked with ghosts assumed the titer of ghosts to be equal to the phage titer before osmotic shock (13, 40). Herriott and Barlow (51) developed an assay based on the lytic property of the ghost, but this was calibrated to the number of phage before osmotic shock and did not provide an independent measure of ghost particles. Assays based on the number of surviving colony formers are not valid because under certain conditions cells infected with ghosts may recover the ability to form colonies (30, 40).

Duckworth and Bessman (30) developed an assay based on the ability of the ghost to inhibit induced enzyme synthesis (40, 113) whereby a value for the number of inhibiting particles in a ghost suspension may be obtained which is independent of the number of phage present before osmotic shock. The decrease in inducibility of β-galactosidase (as per cent of the uninfected control) with increasing numbers of phage or ghosts is plotted on semilog paper, and the ghost titer is calculated from the 37% survival point at which the number of inhibiting particles is theoretically equal to the number of bacteria.

Purification of Ghosts

Because approximately 1% viable phage are routinely contained in ghost preparations (30, 40, 51), a method by which ghosts can be purified free from contaminating phage would be advantageous. This would be particularly important in any studies of ghost-infected cells involving durations of observation greater than 20 to 30 min, as the residual phage can multiply 100-fold or more during this time. Some workers (40) have used ghost suspensions treated with ultraviolet light to obviate this problem.

Herriott and Barlow developed a method for the partial purification of ghosts, but the method was not designed to separate whole phage from ghosts (51). Ghosts can be separated from contaminating phage by sucrose gradient centrifugation, after which the concentration of plaque formers is less than 0.05% of the ghost titer (29). Only one peak of ghost activity (30) appears in the gradient, and this is well separated from the phage which band at a higher density. An electron

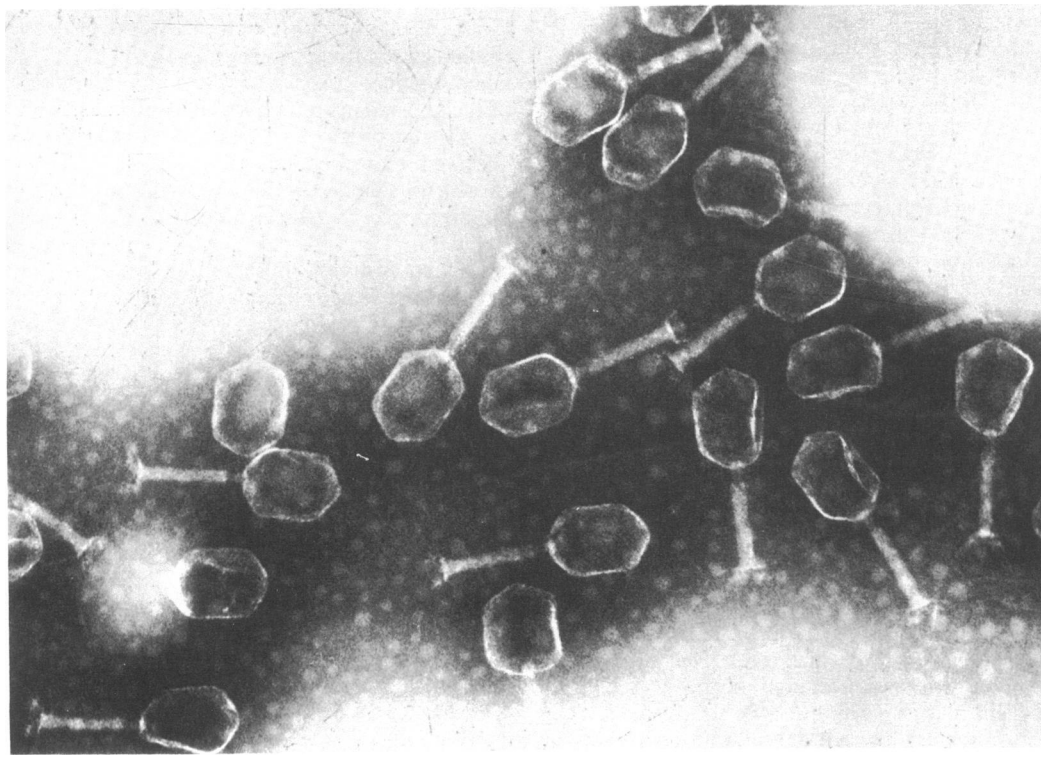

FIG. 1. *T_4 am E957 phage ghosts purified by sucrose gradient centrifugation (29). Phosphotungstic acid negative stain. × 120,000. Courtesy of Carl A. Schnaitman.*

micrograph of ghosts purified in this way is shown in Fig. 1.

Adsorption of Ghosts

French and Siminovitch (40) using ^{35}S-labeled T2H phage and ghosts found that, although 95% of the phage label adsorbed to host cells in 15 min, only 77% of the ghost label was adsorbed during that same time. Herriott and Barlow (51) report that 80% of ^{35}S-labeled ghosts and 95% of ^{35}S-labeled phage are adsorbed. Although 3 to 5% of this difference may be accounted for by the labeled internal protein, there still appears to be 10 to 12% of the labeled protein which is adsorbed as phage but not as ghosts. It is not known whether this represents a fraction of each ghost or a fraction of the population. Hershey (54) observed 90% of ^{35}S-label adsorbed regardless of whether he used phage or ghosts.

Bonifas and Kellenberger (13) studied the adsorption of ghosts by means of electron microscopy and found that at least 99% of the ghosts appeared to be adsorbed. However, they also found that a suspension of phage with a titer of 8×10^{10} plaque-forming units/ml contained only 6×10^{10} ghost particles after osmotic shock as observed in the microscope. Although this is within the error ($\pm 30\%$) of the method, it is possible that some of the phage were so damaged by the osmotic shock that they were not counted as ghosts and remained as unadsorbed counts observed by others (40, 51).

The rate at which ghosts are adsorbed has been shown to be equal to the rate of adsorption of phage (13, 40), and they are not adsorbed to host range mutants which cannot adsorb the parent phage (52).

Antigenicity of Ghosts

No differences of an antigenic nature have been observed between phage and ghosts. Hershey and Chase (55) observed that 99% of the ghosts were precipitated with phage antiserum and that 97% of the phage were precipitated with ghost antiserum. They concluded that ghosts contain the principle antigens of the phage particle.

Lanni and Lanni (72) also observed that shocked preparations of T2 are serologically identical to intact phage, and Herriott and Barlow (51) found that the velocity constants for the reactions of phage or ghosts against four preparations of antisera were the same.

Killer versus Nonkiller Ghosts

There are frequent references in the literature to killer and nonkiller ghosts (13, 40, 52, 75). This distinction arose from the fact that more ghosts appear to be able to adsorb to the host cell than are able to kill (13, 40, 51). Furthermore, there appears to be a discrepancy between the number of ghosts which inhibit some metabolic activity and the number of ghosts which inactivate the colony-forming ability of the cell. To explain this, it has been postulated that there are two kinds of ghosts, one which kills the cell and one which only reversibly inactivates it. However, because the killing ability of a ghost preparation varies with the way the bacteria are grown it seems probable that there is only one kind of ghost which may exert variable effects, depending on the state of the host at the time of infection.

BIOLOGICAL ACTIVITIES OF GHOSTS

Inhibition of Colony Formation

Bonifas and Kellenberger (13) measured the survival of *Escherichia coli* B as a function of multiplicity of T2 phage and T2 ghosts. The phage were diluted so as to equal the concentration of the shocked phage and were added to cells which had been grown in broth and then starved. After 30 min, the cells were plated out to measure surviving colony formers. The survival curves for both the phage and ghosts were single-hit, indicating that one phage or one ghost is sufficient to kill a bacterium. The slopes of the two curves were different, however. Whereas the multiplicity of phage which gave 37% survival was very close to 1, 37% of bacteria survived ghost infection at a multiplicity of 2.7. This indicated that one *active* ghost was produced from 2.7 shocked phage. This observation appears consistently in the literature (28–30, 40, 51, 54, 75). The range of values goes from 1.5 to 5 phage per active ghost, with the average being approximately 2.

Because this number is crucial for the interpretation of some of the earlier work dealing with ghosts, in which the number of ghosts is assumed to be equal to the number of phage before osmotic shock, some time should be spent in consideration of it.

The fact that some cells can recover and form colonies after having been infected with ghosts (30, 40) leads to a great deal of variability in reports on the ability of ghosts to inhibit colony formation. It has been observed, however, that cells grown in nutrient broth and then infected with ghosts do not recover to any detectable level (21, 30). Nonetheless, it is consistently found, even when broth-grown cells are used, that approximately 50% of the cell-killing ability of the phage is inactivated by osmotic shock; i.e., only one active ghost is produced for every two phage that are osmotically shocked. Values taken from Lehman (75) of per cent survival of colony

formation of *E. coli* B grown in nutrient broth also lead to the conclusion that the shocking procedure usually results in the production of only one-half the expected number of active ghosts.

Although French and Siminovitch (40) used cells grown in synthetic media, a high percentage of which recovered after ghost infection (in one experiment in which only 5% of the cells were able to synthesize β-galactosidase after ghost infection, 80% of them were able to produce colonies on overnight plates), their data are not inconsistent with the observation that two phage usually produce one active ghost. In one experiment in which recovery is shown by plotting the number of colony formers with time after infection with a multiplicity of 4.9 ghosts (calculated from the number of phage before osmotic shock), they observed a decrease in cells able to form colonies for 20 min, a constant number for 60 min, and then a normal exponential increase. They interpreted this finding as being consistent with a small number of active ghosts (one-third the number of phage or a multiplicity of ~1.7) and a large fraction of ghost-infected bacteria that recover. However, *assuming* that any uninfected bacteria will continue to multiply at a constant rate, their data are actually more consistent with a rather high multiplicity of active ghosts (between 3 and 4) and a recovery of 20 to 25% of the cells (Fig. 2). Using the value of 3.4 active ghosts out of an input multiplicity of 4.9, we calculate that their ghosting procedure, at least in this case, produces 1 active ghost from 1.4 phage. Using their values for the number of cells which are capable of supporting phage growth after ghost infection, an average value of 1 active ghost from 2.6 phage is obtained (*see* Superinfection Inhibition).

The following conclusions seem, therefore, to be warranted. A population of shocked phage may contain two kinds of particles but contains only one kind of active particle. Although the inactive particles may attach to the bacterial receptor sites, they have no further effect on the cell. On the average, only one-half of the shocked phage become active ghosts. The effect of an active ghost on a cell may vary with the state of the host cell, and although all cells infected with an active ghost are inhibited at least temporarily, some may recover the ability to grow and form colonies. Cells treated in different ways may be rendered more likely to recover from ghost infection, indicating that the reported variability in the killing efficiency of different preparations of ghosts most likely stems from variations in the host cell and not from heterogeneity of the ghost population. The factors which affect the recovery

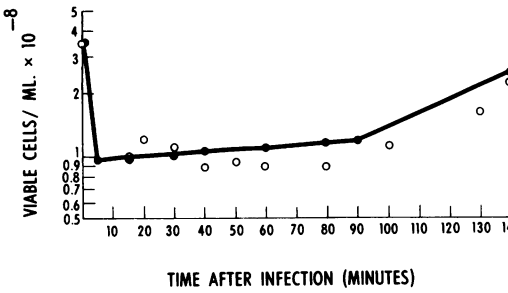

FIG. 2. *Recovery of ghost-infected cells. Open circles are the data of French and Siminovitch (40). Solid circles are calculated on the basis of a multiplicity of 3.4 ghosts per bacterium (3.4% uninfected cells), multiplication of the uninfected cells with a doubling time of 50 min, and recovery of 25% of the cells at 90 min postinfection. Cells which plate as survivors at times between 5 and 90 min are equal to the number of cells which will recover at 90 min plus the number of uninfected cells and their progeny.*

of some cells are largely unknown, except that growth in synthetic media allows a larger proportion of cells to recover (30, 40). The study of these factors may be instrumental in helping to elucidate the mechanism of ghost-induced inhibition of cellular activity.

In light of the above we shall, when a distinction is necessary, refer to a ghost as a shocked phage particle which has been shown to be active in inhibiting some metabolic activity of the host cells such as induced enzyme synthesis or inhibition of superinfecting phage multiplication. The inhibition of colony formation will not be considered a valid index of ghost activity unless it has been specifically shown that the cells in question cannot recover. The term shocked phage will be reserved for those preparations of phage which have been subjected to osmotic shock but whose inhibiting activity has not been determined. Furthermore, we will assume, for purposes of later argument, that only approximately one-half of the shocked phage are ghosts; the other half are inactive as phage or ghosts.

Cell Lysis

Shocked phage preparations contain a factor which causes cell lysis. This lytic factor sediments with the ghost particle, and is not affected by treatment with deoxyribonuclease (51). The lysis of cells by ghosts is in some ways similar to the phenomenon of lysis-from-without caused by very high (>100) multiplicities of phage. Both ghost lysis and lysis-from-without by phage occur more rapidly than the phage lysis seen after a productive infection, and both are inhibited by Mg^{++} (8). Ghost lysis, however, occurs with lower

multiplicities of ghosts (29, 51, 52, 98, 100), with some lysis being observed at multiplicities of 10 or thereabouts.

Lysis is not the primary cause of the inhibition of metabolic activity seen after ghost infection. Cells may be killed but not lyse (29, 30, 52), and 0.05 M spermidine which inhibits the lysis of cells by ghosts (18) does not prevent the ghost-induced inhibition of induced enzyme synthesis (D. Duckworth, *unpublished data*). All of the factors which affect the lysis of cells by ghosts are not known, although multiplicity, length of time between addition of ghosts and measurement of lysis, and ion content of the medium are all important (8, 18, 51, 52, 98).

Superinfection Inhibition

If cells are infected with ghosts for durations ranging from 5 to 100 min and then T1 or T2 phage is added, virtually no multiplication of the superinfecting phage occurs (13, 40, 52). If the phage are added at 100 min after ghost infection (40), the number of bacteria which will produce an infective center is very nearly equal to the number of cells which plate as survivors at 7 to 15 min after ghost infection. As these are seen to be predominantly cells which were inactivated but recovered on the plates, we may conclude that about 100 min after ghost infection some proportion of the cells recover the ability to support the multiplication of superinfecting phage. If phage are added 150 min after ghost infection, the number of cells forming an infective center are approximately double what they were at 100 min, indicating that the cells also recover the ability to multiply.

If one measures the proportion of ghost-infected cells which can support phage multiplication at 7 to 15 min after the addition of the ghosts, one can determine the proportion of cells which are both reversibly and irreversibly inactivated. Using multiplicities of shocked phage of 7.1, 6.0, and 6.3, French and Siminovitch (40) observed that 2.5, 3, and 2%, respectively, of the cells will produce infective centers if these are measured before 15 min after the addition of the ghosts. These numbers were used to calculate the "1 ghost from 2.6 phage" mentioned previously.

Herriott and Barlow (52) looked for inhibition of phage multiplication by ghosts when the ghosts and phage were added simultaneously. They observed that when the ghosts and phage were added to cells in nutrient broth, one-third to one-fourth of the added ghosts caused inhibition of infective-center formation. A larger percentage of the shocked phage caused inhibition when added prior to the phage. When the phage and ghosts were added simultaneously to starved cells and then placed in a nutrient medium, there was very little interference by the ghosts. These results are quite difficult to interpret, however, as the relative multiplicities of phage and ghosts are not given, and this would affect the results considerably. The lack of interference in starved cells is doubly hard to interpret because only 30 to 50% of the added phage form infective centers even in the absence of ghosts (10, 46, 52).

Bacteria infected with T2 phage after having adsorbed ghosts do not show the characteristic transformation of the nucleus, that is, the formation of marginal vacuoles, which is seen after a productive phage infection (82, 87). Rather, the nuclei retain the diffuse or vesicular appearance characteristic of ghost-infected cells (13). The inability of ghost-infected cells to produce phage is apparently not due to lysis of the cells by ghosts (13, 40, 52) or to the breakdown of the superinfecting phage DNA (38, 39, 45).

Energy Metabolism in Ghost-Infected Cells

When a culture of bacteria is infected with phage, the exponential rate of oxygen consumption seen in uninfected cells becomes linear (25), presumably because the synthesis of components in the respiratory chain has ceased, while leaving the function of previously synthesized ones unimpaired. French and Siminovitch (40) measured oxygen consumption in ghost-infected cells using shocked phage multiplicities of 4 and 8 and found the rates to be 37 and 10%, respectively, of the phage-infected control. (Phage-infected cells were used as a control due to the exponential increase in O_2 consumption observed in uninfected cells. As neither the phage-infected nor the ghost-infected cells are multiplying, and as respiration is not inhibited in phage-infected cells, the comparison is a valid one and obviates the problem of converting to a "per cell" basis.) If these multiplicities represented the actual multiplicity of active ghosts and if O_2 consumption were completely inhibited in ghost-infected cells, we would expect the rates to drop to 1.8 and <0.1%. If, as is more probably the case, only 1 ghost is produced from every 2 phage (*see* Inhibition of Colony Formation), the actual multiplicity of active ghosts would be 2 and 4 and the expected consumption of oxygen would be 13.5 and 1.8%, respectively. Because they observe considerably more oxygen consumption than this, we conclude that respiration may be impaired in ghost-infected cells but is not completely inhibited. Table 1 illustrates this along with other observations of French and Siminovitch. The fact that any inhibition of O_2 consumption is seen may be due to lysis of some proportion of the cells. Lehman and Herriott (76) made this suggestion to explain

TABLE 1. *Biological activity of ghosts*

Activity	Ghost multiplicity[a]	Theoretical per cent survival[b]	Experimental per cent survival[c, d]	Per cent survival of colony formation[d]
Colony formation	2.5	8.0	30.0	30.0
Colony formation	2.4	9.0	26.0	26.0
Superinfection exclusion	3.5	3.0	7.8	37.0
Superinfection exclusion	3.0	5.0	7.3	56.0
Superinfection exclusion	3.2	4.0	7.1	68.0
Oxygen uptake	2.0	13.5	37.0	
^{35}S uptake	2.6	7.0	12.0	55.0
^{32}P uptake	2.4	9.0	36.0	53.0
β-Galactosidase synthesis	2.8	6.0	5.0	80.0

[a] Values represent the number of ghosts added per bacterium, assuming that one-half of the shocked phage are inactive as phage or ghosts.

[b] Calculated from the Poisson distribution which states that $P(o) = e^{-n}$, where $P(o)$ is the proportion of uninfected cells and n is the multiplicity.

[c] Per cent of the measured activity which is found in the ghost-infected cells, as compared to the uninfected (or in the case of oxygen uptake, the phage-infected) control.

[d] Values were taken from reference 40.

their different findings. They measured cell respiration in ghost-infected cells in synthetic medium [as did French and Siminovitch (40)] and found that on a per (unlysed) cell basis the oxygen consumption in cells multiply infected with ghosts was not significantly different from that of uninfected or phage infected cells. However, because they based their correction for cell lysis on a measurement of turbidity alone their conclusion may not be entirely warranted, as a drop in turbidity could come from a change in shape of the cells and is not necessarily indicative of lysis. Lehman's (75) measurement of O_2 consumption by cells grown in broth and then infected with ghosts also supports the conclusion that respiration is not markedly inhibited. Respiratory quotients (micromoles of CO_2 evolved per micromole of O_2 consumed) are also the same in normal, phage-infected, or ghost-infected cells (76).

Inhibition of Host Macromolecular Syntheses

The synthesis of induced enzymes is very efficiently inhibited by ghost infection (40, 113). This is not surprising in light of the marked effects which ghosts have on host macromolecular syntheses. If cells are grown in nutrient broth and then infected with ghosts, DNA synthesis, ribonucleic acid (RNA) synthesis, protein synthesis, β-galactosidase induction, and colony formation are all inhibited with one-hit kinetics, with one ghost, as previously defined, sufficing to inhibit all measured syntheses (29). If the cells are grown in synthetic media and then infected with ghosts, protein synthesis is inhibited to a degree which is slightly less than the inhibition of β-galactosidase induction, whereas RNA synthesis and colony formation are inhibited to an extent less than either β-galactosidase induction or protein synthesis (Fig. 3). None of the inhibitions deviates significantly from one-hit kinetics. These results substantiate the earlier observations of French and Siminovitch (40) which are summarized in Table 1, although Herriott and Barlow (52) and Lehman (75) concluded that the synthesis of RNA in ghost-infected cells is more susceptible to ghost infection than DNA or protein synthesis. The differences may be due to the techniques involved or to the prior history of the cells, or both.

The inhibition of nucleic acid synthesis is not due to a block in the assimilation of nitrogen or phosphorous or to the inhibition of synthesis of organic phosphates (76). The majority of the phosphorous which is assimilated is found in organic phosphorous compounds in the medium, however. The preexisting pools of organic phosphates and material absorbing at 260 nm, as well as any ^{32}P assimilated and esterified after infection, all accumulate in the medium (75, 76).

In summary, it has been seen that when cells are grown in nutrient broth and then infected with ghosts all macromolecular syntheses stop in every cell infected with a single ghost, and none (or very few) of the cells recovers or forms a colony; when cells are grown in synthetic media and then infected with ghosts, the majority react as do the nutrient broth-grown cells. Some cells, perhaps all those in one particular phase of the growth cycle, stop synthesizing induced enzymes and synthesize little protein and only some RNA. They probably either make no messenger RNA or cannot initiate translation of any made during the course of the infection; these cells later recover

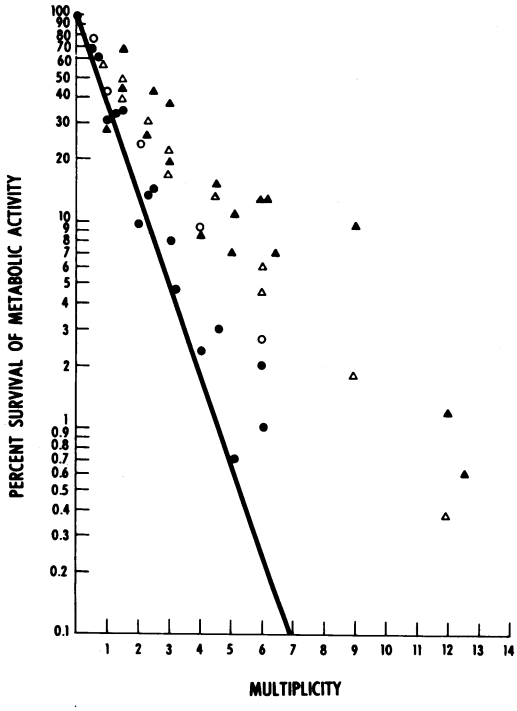

FIG. 3. *Inhibition of metabolic activity by ghosts at various multiplicities of infection in synthetic media. E. coli B was grown to a concentration of 4 × 10⁸ cells/ml in M-9 medium (76) + 0.5% glycerol. L-Tryptophan (100 μg/ml) was added prior to the addition of T4 am E957 phage ghosts. After 2 min of infection, inducer of β-galactosidase (5 × 10⁻⁴ M isopropyl-β-D-thiogalactopyranoside) was added simultaneously with either ¹⁴C-uracil (1 μCi/ml, 10 mCi/mmole) or ¹⁴C-D-L-leucine (1 μCi/ml, 10 mCi/mmole). After 10 min, the cells were chilled, washed, resuspended and assayed for either β-galactosidase or acid-precipitable radioactivity (29). A sample of the chilled cells was plated to measure the number of colony formers. Results are plotted as the per cent of the uninfected controls. Ghost titer was determined as previously described (30). The solid line is the theoretical per cent survival based on the Poisson distribution (2). Symbols: ●, β-galactosidase synthesis; ○, protein synthesis as measured by incorporation of ¹⁴C-D-L-leucine; △, nucleic acid synthesis, as measured by the incorporation of ¹⁴C uracil; and ▲, colony formation.*

from the effect of the ghost and appear as colonies on overnight plates. It is possible that the relative extents of nucleic acid and protein synthesized in these reversibly inhibited cells may vary from culture to culture or even from cell to cell.

One possible reason that macromolecular syntheses cease in ghost-infected cells is that the transport of precursors into the cell is inhibited. To test this, the uptake by ghost-infected cells of a variety of nucleic acid and protein precursors has been measured. Using broth-grown *E. coli* B, no detectable accumulation of labeled uracil, uridine, adenine, guanine, cytosine, D-L-leucine, or D-alanine was observed that could not be accounted for by the presence of uninfected cells; thymidine transport is also greatly inhibited. The ghost-infected cells can, however, transport some sugars (H. Winkler and D. Duckworth, *unpublished data*) and can assimilate P_i and ammonia (76).

Whether the lack of transport causes the lack of macromolecular syntheses, or vice versa, cannot be determined from these experiments; however, it was observed that phage-infected cells do transport thymidine at the same rate regardless of whether they are synthesizing DNA. This was observed using an amber mutant of T4 phage, T4 am E957, which is defective in the deoxynucleotide kinase gene and cannot, therefore, synthesize any phage DNA when used to infect *E. coli* B. The synthesis of host DNA is inhibited by this phage so that it was possible to observe the transport of thymidine into the cells in the absence of any DNA synthesis. Thymidine transport was not inhibited for up to 8 min after phage infection, although it was at least 90% inhibited in the ghost-infected cells. This transport in the phage-infected cell is not dependent on protein synthesis because it also occurs when T4 am E957 is used to infect chloramphenicol-pretreated cells. In this case, however, some synthesis of host DNA occurs (29).

Inhibition of Colicin Activity

Bacterial DNA is broken down after the infection of a susceptible cell by colicin E2. This is apparently caused by a change of state of the DNA which is effected by the colicin through the cell membrane (90, 91). The colicin-induced degradation of the DNA can be prevented or stopped in mid-course by the addition of T-even phage (B. Swift and J. Wiberg, *personal communication*). These workers also found that T4 phage ghosts inhibit the E2-induced degradation of host DNA. A multiplicity of 5 shocked phage in the presence or absence of 200 μg of chloramphenicol per ml is as effective as a multiplicity of 15 intact phage in the presence of chloramphenicol. With intact phage the presence of chloramphenicol is necessary to prevent the phage-induced breakdown of the host DNA. The greater efficiency of ghosts in causing the inhibition of breakdown is not due to more efficient lysis of the cells by ghosts, as concentrations of Mg^{++} which inhibit ghost-induced lysis do not prevent the ghost-induced inhibition of E2-stimulated DNA breakdown.

Effect of Chloramphenicol on Ghost Infection

Chloramphenicol (100 μg/ml) has been shown to partially protect cells from the phage shut-off of macromolecular syntheses (29, 96). It does not, however, have an equal ability to prevent the inhibition of macromolecular syntheses by ghosts. No RNA synthesis is observed in chloramphenicol-pretreated ghost-infected cells that cannot be accounted for by the number of survivors. The chloramphenicol-pretreated cells cannot transport uracil, nor is the leakage of uracil containing compounds which occurs after ghost infection prevented by the chloramphenicol (29). These results indicate that protein synthesis does not need to occur for the inhibition of host functions and cell death after ghost infection. As indicated in the previous section, chloramphenicol also does not prevent the ghost-induced inhibition of colicin E2 activity.

What Substructures Cause Killing?

Only those phage which are able to adsorb to bacteria can kill them (134). It is possible, however, that a phage or ghost may adsorb to a cell without the subsequent death of the cell (13, 40, 110, 133, 134), indicating that the tail fibers are not responsible for cell death. In trying to isolate other components which may be responsible for killing, one is therefore faced with the problem of isolating the killing component without disturbing the adsorption mechanism. Any process of disruption of the phage that might affect both could not be used. There is, in other words, a problem of "geometry." It is not surprising, therefore, that the only subphage particles isolated (so far) that can kill cells are ghosts. Several attempts have been made to fractionate ghosts, but without success (D. Duckworth, *unpublished data*). The reason for this may be the above-mentioned problem of geometry.

Some speculation has been made, however, about the killing component from studies on the irradiation of phages. Studies with several wavelengths of ultraviolet light (134), X-rays (74, 133), and deuterons and alpha particles (110) have led to the conclusion that the phage has more than one mechanism for inactivating bacteria, only one of which is associated with the DNA. The other is apparently associated with the protein coat and has been reported to have a molecular weight of 90,000 (74), or 1.5×10^6 (110).

INHIBITION OF HOST SYNTHESES AFTER PHAGE INFECTION

Kinetics of Inhibition

It has been stated frequently (5, 24, 119, 132) that all host macromolecular syntheses come to a sudden and complete halt after T-even phage infection. The evidence in support of this is as follows.

(i) A wide variety of inducible host enzymes is not synthesized after phage infection, and components of the respiratory chain do not increase (11, 25, 79, 84); in the case of β-galactosidase, simultaneous addition of enzyme inducer and phage to a culture results in a complete block of the synthesis of the enzyme (11, 64–66).

(ii) Net synthesis of RNA is not detected after phage infection, (19, 23, 53, 69, 127), but if radioactive label is added to the culture several minutes after infection, label is incorporated into an RNA fraction (6, 17, 53, 92, 127, 128); the specific activity of this fraction remains constant after 10 min postinfection, and it has a base composition which mimics phage DNA and not host bulk RNA or DNA (6, 127, 128) and is complementary to phage DNA (47, 48).

(iii) No wholly new ribosomes are synthesized after infection, and phage proteins are synthesized on ribosomes which existed in the cell prior to infection (17, 48).

(iv) Host DNA synthesis cannot be detected for approximately 5 min after infection, at which time the synthesis of phage DNA begins (19, 23, 56, 123).

More recently, the synthesis of host macromolecules has been examined at very early times after infection and it has been seen that some host syntheses can occur for up to 5 min after infection (49, 62, 66, 71). The shut-off of host DNA synthesis has been seen to lag about 1 min behind the injection of the phage DNA (66), and phage-infected cells incorporate short pulses of ^{32}P into RNA which anneals specifically to host DNA (49, 66, 71). It has been reported that host RNA represents 60% or more of the total RNA synthesized during the first several minutes (49, 66) and 13 to 50% of that synthesized from 3 to 5 min after infection (66, 71). Kennell (66) observed that transfer RNA, ribosomal RNA, and messenger RNA are all synthesized at about the same rates relative to each other as they are in uninfected cells, although Landy and Spiegelman (71) concluded that the host RNA that is labeled after infection contains less messenger RNA than that labeled during a comparable period in uninfected cells. Host protein synthesis in infected cells has been studied by disc gel electrophoresis of extracts of cells which had been pulse labeled with amino acids before and at various times after infection; autoradiography of the gels shows that from 1 to 3 min after infection host proteins are labeled at a level approaching 25% of the uninfected control value. Host proteins are not, however, labeled after 3 min postinfection. A

wide variety of mutants of phage including some which cannot degrade host DNA all have the same effect on the shut-off of host protein synthesis (62). Although the synthesis of host DNA after infection would seem to be the easiest of all syntheses to measure due to the difference in base composition between host and phage DNA, no one has studied the kinetics of the shut-off of host DNA synthesis. Phage mutants which cannot synthesize phage DNA would be very useful for studying this problem. Warner and Hobbs (130) studied DNA synthesis in a variety of these mutants, but did not look at very early times.

Is the Inhibition Due to Breakdown of the Host Chromosome?

Although complete inhibition of host syntheses by the T-even phage is perhaps not immediate, the host chromosome does become nonfunctional before gross physical damage has occurred. Kutter and Wiberg (70) and Bose and Warren (14, 131) studied this problem and observed that the breakdown of host DNA occurs in at least two stages, the latter stage (acid solubilization) being controlled by phage genes 46 and 47. Although there are only very slight changes in the sedimentation of the host chromosome during the first 5 min after infection (and these changes not necessarily due to degradation), some damage very early in infection cannot be ruled out. However, this is technically very difficult to measure due to the fragility of the host chromosome, even in uninfected cells, and an inability to recover all of the DNA from infected cells at very early times after infection (70).

Mutants in genes 46 and 47 which cannot completely degrade the host chromosome, or ultraviolet-irradiated phage which have lost this function, retain their ability to inhibit host functions (60, 62, 70, 131). Sadowski and Hurwitz (104, 105) recently described two endonucleases which are induced after T4 infection and act on *E. coli* DNA but not on T4 DNA, but it is not yet known what effects mutations in the genes for these enzymes have on the inhibition of host syntheses.

Nomura et al. (94) showed that phage-infected Hfr males could transfer functional markers to recipient bacteria for 10 to 20 min after infection, providing the mating was begun before infection, indicating that the cessation of host functions is not due to irreversible damage to the chromosome, at least before 10 min postinfection. They did, however, observe a greater transfer of functional DNA in the presence of streptomycin, as would be expected.

Is Protein Synthesis Necessary?

There is a great deal of debate as to whether protein synthesis is necessary for the inhibition of host syntheses after infection. Cohen, in one of the pioneering studies on the metabolism of phage-infected cells (23), showed that the addition of 5-methyltryptophan to T2-infected cells prevented the cells from synthesizing any DNA, although they did so rapidly in the absence of the inhibitor. Net synthesis of RNA was not observed in infected cells either in the presence or absence of this inhibitor, although it could be readily detected in the uninfected cells. Burton (19) also could not detect any DNA synthesis in T2-infected cells in the presence of 5-methyltryptophan. He also showed that two bacterial mutants, one which required tyrosine for growth and one which required tryptophan, when infected in the absence of the required amino acid did not synthesize any DNA.

In the studies described above, colorimetric procedures were employed which may not have been sensitive enough to detect a low level of synthesis. Studies with radioisotopes, however, often show that host syntheses are inhibited even when normal protein synthesis cannot occur. Volkin (126) measured the incorporation of ^{32}P into DNA and RNA in an adenine- and arginine-requiring mutant and showed that, when these cells were deprived of arginine and then infected with T2, they incorporated into DNA only 3% of the number of counts incorporated by the uninfected control in 20 min. The incorporation of ^{32}P into RNA in these experiments was only inhibited about 40%. Cohen and Ennis (22), using a K^+-deficient mutant, showed that there was no DNA synthesis in phage-infected cells in K^+-free media, a condition which inhibits protein synthesis in these cells. The uninfected cells in this same medium apparently do synthesize some DNA (H. L. Ennis, *personal communication*).

Nomura et al. (94) showed that pretreatment of *E. coli* B with 80 μg of chloramphenicol per ml or 250 μg of streptomycin per ml prevented DNA synthesis (host or phage) in the infected cell when a multiplicity of 10 was used. Tomizawa and Sunakawa (123) and Astrachan and Volkin (7) also showed that addition of chloramphenicol at the time of infection prevented DNA synthesis.

There is some, but not complete, inhibition of host RNA synthesis in infected cells in which protein synthesis is inhibited by several different methods. Ennis and Cohen (33) used a mutant of *E. coli* B which cannot concentrate K^+ or synthesize protein in the absence of external K^+ and found that, after infection with T4 in K^+-free media, host ribosomal, messenger, and transfer

RNA are all synthesized but in reduced amounts as compared to the uninfected cell. Phage messenger RNA is seen to be synthesized simultaneously with the host RNA made, confirming the results of others (49, 93, 96, 97). Volkin (126) found that RNA was made when an arginine auxotroph was infected in the absence of arginine, but the contribution of host RNA to this appeared to be small, judging by the base composition. Various workers have reported the synthesis of some host RNA in the presence of chloramphenicol (7, 49, 93, 96, 97).

Host messenger RNA synthesis may be inhibited preferentially (93, 97) in the presence of chloramphenicol as it appears to be in the presence of streptomycin (94) and in the absence of a required amino acid (109). When phage infect puromycin-treated cells, there is no loss of the ability of the phage to inhibit the synthesis of induced β-galactosidase (64), indicating no effect of puromycin on the shut-off of host syntheses.

The effect of chloramphenicol on the inhibition of host nucleic acid synthesis by T4 at various multiplicities of infection has been investigated by Nomura et al. (96). They concluded that both host DNA and host-specific messenger RNA are synthesized in chloramphenicol-pretreated cells, in addition to ribosomal and soluble RNA, but that all nucleic acid synthesis is inhibited and the degree of inhibition becomes higher at higher multiplicities of infection and at longer times after infection.

To summarize, in the absence of protein synthesis, phage infection causes either the complete inhibition of host messenger RNA synthesis or incomplete inhibition of the messenger synthesis with complete inhibition of the initiation of its translation (or both), but incomplete inhibition of the synthesis of host ribosomal and transfer RNA (7, 33, 49, 64, 93, 96, 97, 109). DNA synthesis is inhibited by phage in cells which cannot synthesize normal protein due to the presence of streptomycin (94) or to a lack of K^+ (22) or some essential amino acid (19, 23, 126); host DNA can, however, by synthesized when protein synthesis is inhibited by chloramphenicol. In the latter case, various effects are noted (7, 19, 22, 23, 29, 94, 96, 97, 123, 126). The variation is not just a matter of multiplicity because Tomizawa and Sunakawa (123) used a multiplicity of 5, at which Nomura et al. (96) observed 50% synthesis, and did not observe any DNA synthesis when the chloramphenicol was added simultaneously with the phage. The differences may be due to length of treatment with chloramphenicol, concentration of chloramphenicol, or the time after infection at which the measurements are made.

When a mutant phage which cannot synthesize any phage DNA (T4 am E957) is used (at a multiplicity of 5) to infect cells which have been pretreated for 10 min with 100 μg of chloramphenicol per ml, it is found that DNA synthesis proceeds at about 50% the rate observed in uninfected cells for at least 8 min (29). J. D. Karam (*personal communication*) observed similar effects using other DNA-negative mutants of T4. There is no DNA synthesis in the infected cells in the absence of chloramphenicol. Cohen (24) suggested that chloramphenicol may have effects other than its effect on protein synthesis, a suggestion which seems plausible in light of the fact that host DNA synthesis is not observed when amino acid auxotrophs are deprived of their required amino acid and then infected (19, 23, 126). Silver et al. (116) observed that chloramphenicol stimulates the influx of potassium into uninfected cells, an observation not readily explained by the effect of chloramphenicol on protein synthesis, and Lark and Lark (73) also noted some unusual results using chloramphenicol. Alternatively, of all the inhibitors tested chloramphenicol may be the only one which completely inhibits protein synthesis.

The multiplicity effect observed by Nomura et al. (96) led these workers to postulate that there are two mechanisms of T4 inhibition of host nucleic acid synthesis. The first mechanism is dependent on phage protein synthesis and the second one is independent of protein synthesis but dependent on multiplicity. Terzi and Levinthal (120, 121) have given some support to this, although Karam (*personal communication*) reports that there is virtually no effect of multiplicity if 300 μg of chloramphenicol per ml is used. Nomura et al. (96) do not exclude the possibility that their multiplicity effect is due to a preferential escape of phage gene function from chloramphenicol inhibition at higher multiplicities.

It is noteworthy that some inhibition of host syntheses (from 50 to 90%) is reported in all cases of infection in the absence of protein synthesis.

CELL SURFACE CHANGES DURING PHAGE AND GHOST INFECTION

Leakage of Metabolites During Phage Infection

Puck and Lee (99, 100) were the first workers to study the leakage of metabolites from phage-infected cells. They found that if cells are labeled for several hours with ^{32}P or ^{35}S and then infected with phage, various amounts of radioactivity are released into the medium. The factors which effect this leakage are not entirely clear, but it

appears as if the maximal amount of radioactivity is released within several (3 to 5) minutes after infection and that superinfection with homologous phage after 8 min does not cause any further leakage; the leakage is independent of multiplicity, although with qualifications—for instance, larger molecules appear in the medium if the multiplicity is raised. They found that as much as 20% of the ^{32}P in cells is caused to leak out by the phage using multiplicities from 1 to 3.5, and they said that because only a small proportion of the potentially available material was liberated some "sealing" agency must have been operative. The fact that the reaction appears to stop at about 5 min postinfection would tend to substantiate this. However, the fact that the reaction stops before all of the ^{32}P has been released in no way indicates that there is more *available* material that has been prevented from leaking out; it could simply mean that the cell has become permeable to only certain compounds and that as phage infection progresses these compounds are depleted.

Other workers have found that ^{32}P from prelabeled cells appears in the medium after phage infection, although no one has found the very high percentages that Puck and Lee (99, 100) found. Sechaud et al. (112) found that 2% of the ^{32}P-containing material was released from extensively labeled cells within 10 min after phage infection when cells were infected with a multiplicity of 5 T4 phage, and Silver et al. (116) reported leakage of about 5% of ^{32}P label after 30 min of infection with T2 phage at a multiplicity of either 3.1 or 6.2; 0.9% of the label appeared in the medium at very early times.

The reasons for the much higher values of leakage reported by Puck and Lee (99, 100) are not entirely clear, although they themselves note a great deal of variation from experiment to experiment. Their use of cells which had been stored for as much as 5 days and their own observation that old cells leaked substantially more ^{32}P than fresh cells may account for the variation, however. Silver et al. (116) found that their stock of T6 at a multiplicity of 3 caused a greater leakage than their stock of T2 at the same multiplicity, and suggested that this could be due to a larger number of "dead" phage particles in the T6 stock. As several workers (29, 98) have observed that ghosts cause more leakage than phage, this is entirely possible.

The leakage of several nucleic acid derivatives from cells following infection by phage T4 has been studied, and it has been found that when the cells are prelabeled for 5 min with uracil, uridine, adenine, or cytosine from 3 to 8% of the total counts of the cells are released within 8 min following phage infection. The exact contribution that lysed cells make to these figures was not determined. Ghosts, on the other hand, cause the release of up to 30% of the counts, usually within 2 min (29).

Silver et al. (116) studied the leakage of potassium ion from phage-infected cells and found that this ion leaks out of the cell for about 5 min postinfection but not thereafter. They postulate that the leakage is due to cell surface damage by the phage and that the damage is subsequently repaired to prevent further loss. As the leakage was stopped even when ultraviolet-irradiated phage were used or when chloramphenicol was present, the repair is attributed to host enzymes. An alternate hypothesis that was not considered is that the K^+ is being replaced, at least in part, by some ions injected by the phage. This would explain the multiplicity effect which was observed and would be substantiated by the fact that excess nonradioactive potassium causes a renewed release of the radioactive K^+, even after the phage appears to have resealed the surface. It is known that the cation content of some osmotic shock-resistant mutants can be altered by altering their external environment (3, 16). It would be interesting to look at the potassium release from cells infected with phage differing in their cation content.

Putrescine is observed to leak out of r^+-infected K-12(λ) cells for about 8 min and then stop. It may even be reabsorbed (24, 34). In rII-infected cells, putrescine continues to be released and this may be why the rII infections become abortive. Bode observed that adenosine triphosphate was released from K-12(λ) r^+-infected cells at a constant rate for 15 min or more (12), although Fields did not observe the same leakage from *Shigella* when it was infected with T2 (35).

Leakage of Metabolites During Ghost Infection

Prater (98), studying the leakage of 260 nm absorbing material from phage-infected cells, found that when ghosts are used instead of phage three to four times as much material is released. In both cases, the material was not sedimented at 50,000 × *g* in 1 hr and was dialyzable, but whether the compounds released in both cases were identical is not known.

Herriott and Lehman and their co-workers (52, 76) observed that inorganic phosphate is taken up by ghost-infected cells, becomes organically bound, and is then discharged back into the medium. The possibility that the phosphate was becoming organically bound by enzymes in the media which may have leaked out of the

ghost-infected cell was discounted by the finding that the supernatant fluid from a ghost-infected culture did not catalyze the incorporation of the phosphate into an organic linkage. So apparently the phosphate is transported into the cell, undergoes a series of reactions, and is then transported or leaks back out into the media in a different form.

When cells are prelabeled with a variety of nucleosides or nucleotide bases and then infected with ghosts, from 14 to 30% of the label is found in the medium within 8 min and usually before 5 min. The reaction is initially very rapid and then levels off. In this respect, it appears as if some "sealing" reaction is taking place. However, the appearance of radioactivity in the medium parallels the loss of soluble counts from the cell, and if the cells are pulse labeled and the label "chased" before ghost infection, there is very little leakage. The majority of the counts in the medium are acid soluble, but some precipitable material is present (29). Because the appearance of radioactivity in the medium could come from cell lysis and because ghosts have the ability to lyse their host cells (51, 52), the pools of acid-soluble nucleotides in the cells were studied.

It was found that after ghost infection, usually within 2 min, the acid-soluble pools were completely depleted and the counts appeared almost quantitatively in the surrounding media (29). No breakdown of nucleic acid could be detected, in agreement with Lehman and Herriott (76). In phage-infected cells, the counts in the acid-soluble pools remained at almost the same level for from 15 sec to 5 min after infection. The fact that the radioactivity seen in the medium after phage infection does not appear to come from the soluble pools of the cells may be because the radioactivity results from some specific external hydrolytic reaction or because it results from a few lysed cells, although some workers have discounted the latter possibility (98–100). Losses from the soluble pools of the cells during the first 15 sec after phage infection are also not ruled out.

The fact that ghost-infected cells have been seen to release from three to five times the amount of metabolites that are released from phage-infected cells has been interpreted as meaning that the ghosts cannot repair the damage attendant upon the process of injection and that ghost-infected cells become nonviable because of the lack of the repair mechanism (81, 99, 100, 122). The fact that the loss of material from the acid-soluble pools in phage-infected cells never parallels the loss from ghost-infected cells does not support this concept of damage which is repaired by phage-induced enzymes. The possibility that the pools in phage-infected cells are otherwise stabilized cannot be completely ruled out, however, until studies on the composition of the pool and the specific activities of the compounds found therein are performed.

Effect of Mg^{++}

It was mentioned earlier that Mg^{++} can suppress lysis from without by phage (8, 100) and the lysis of cells by ghosts (8); 0.05 M spermidine also prevents ghost lysis (18). Puck and Lee (99) observed that 0.025 M $MgCl_2$ inhibits the leakage of ^{35}S after phage infection by about 85% but does not inhibit adsorption or injection of DNA, and Silver et al. (116) showed that Mg^{++} can inhibit the leakage of K^+ from phage-infected cells. The effect of Mg^{++} on the leakage of metabolites from ghost-infected cells has not been studied, although in one experiment we did not observe a very great effect on the release of ^{14}C-labeled uracil compounds from ghost-infected cells. At a concentration of 0.025 M (the highest concentration used) the reaction was inhibited only 15%. Spermidine does not prevent the inhibition of induced enzyme synthesis by ghosts, as previously mentioned (D. Duckworth, *unpublished data*).

Much higher concentrations of Mg^{++} (0.2 M) can inhibit cell killing by both phage and ghosts, although in the case of these very high concentrations there is some effect on adsorption (8). Mg^{++} is also instrumental in preventing abortive infections in K-12(λ) by rII phage (44, 108).

The effect of Mg^{++} and the organic cations is a subject which deserves special attention. It may be that these ions have some specific, as yet undetermined, effect on the integrity of the functional contact between the membrane and the macromolecular synthesizing systems that would explain their varied effects on phage and ghost infections. Changes in these ions cannot, however, be totally responsible for the observed effects of infection, as these ions do not prevent all changes that occur. In this regard, the observations of Shalitin and Katchalski (111) on the effects of poly-L-lysine are interesting.

Transport in Phage and Ghost Infection

The effects of phage and ghosts on some transport systems have been mentioned in previous discussions of inhibition of host syntheses by ghosts and of leakage. In summary, it was found that ghosts inhibited the accumulation of leucine and several nucleic acid precursors by the cells (29), but not the uptake of P_i or NH_3 (52, 76). Phage do not affect the transport of nucleic acid precursors even when they are unable to synthesize any DNA (29). Luria (80) and Fields (35)

reported that the transport of several substances is not affected in phage-infected cells, unless the phage has infected the cell abortively, and Silver et al. (116) observed that the influx of Mg^{++} and K^{+} is not blocked by phage infection. Preliminary work on the transport of sugars during phage and ghost infection has shown that the ability of the cell to accumulate several sugars is somewhat affected after phage and ghost infection, but to varying extents which are different for different sugars (H. Winkler and D. Duckworth, *unpublished data*).

Role of Lysozyme

For many years it was thought that lysozyme was bound to the phage tail (9) and thereby assisted in the injection of the DNA. The leakage from ghost-infected cells has been attributed to this lysozyme (51, 81). Using phage which have a mutation in the lysozyme gene, Emrich and Streisinger (32) showed that the enzyme coded for by this gene has no role whatsoever in the initiation of infection or in lysis from without. The product of the lysozyme gene also has no role in the biological activity of phage ghosts (28). Whether there are other genes which code for lytic enzymes which could be responsible for the injection of DNA and the "leakiness" observed after phage and ghost infection has not been determined. Lytic enzymes are not necessary, however, for the loss of metabolites from bacteria, as it has been reported that various small molecules can cause changes in the cell surface that lead to specific losses of metabolites from bacteria (43, 89, 106).

Other Membrane-Associated Phenomena in Phage Infection

If phage-infected cells are superinfected 2 min or more after the initial infection, the superinfecting phage do not contribute genetic markers to the progeny (26). The DNA of the superinfecting phage remains at the outer surface of the cell (100) where it is broken down (38, 39, 45). A primary infection with heavily irradiated phage stimulates the exclusion of superinfecting phage, indicating that phage enzymes may not be involved (26). These observations and the related observation of Visconti (125) on the resistance to lysis-from-without of phage-infected cells indicate that the membranes of *E. coli* cells may be altered by T-even phage infection. The phenomenon of lysis inhibition which occurs with r^{+} phage is also probably related to changes in the cell membrane (12, 27, 34, 44, 85, 108, 112).

Phage-infected cells also have an increased permeability for reduced nicotinamide adenine dinucleotide (102) and acridine dyes (36, 58, 114) and are more sensitive to growth inhibition by these dyes than are their host. Mutations in the *pr* gene in T2 or the *ac* gene in T4 are much more resistant to this growth inhibition than are wild-type T2 and T4, although the mutants are still more sensitive than *E. coli* (57, 114, 115). The mutations have been shown to cause a reduced uptake of acridines in the infected cells relative to cells infected with sensitive phage (114, 115).

Chloramphenicol pretreatment of the cells or extensive ultraviolet irradiation of the phage only partially prevent the increased permeability and do not appear to affect the initial rate of uptake. Ghost-infected cells act as do the ultraviolet-irradiated phage-infected cells in that they take up some dye but not as much as normal phage-infected cells. Chloramphenicol (25 μg/ml) does, however, abolish the protective function of the *pr* gene (114).

Silver (115) suggested that the *pr* gene product affects the cell membrane in such a way as to render the cells permeable to the dye during the entire course of infection. Mutations in the *pr* gene do not, however, completely abolish the enhanced dye uptake by infected cells (114), and the mutations can be overcome by very high concentrations (4 μg/ml) of dye (58). Mutations at several sites in the early enzyme region of the T2 map can suppress mutations in the *pr* gene (59).

There are also changes in phospholipid synthesis after T4 phage infection. It has been found that the rate of incorporation of P_i into phospholipids is decreased and that the relative rates of synthesis of phosphatidyl-glycerol (PG) and phosphatidylethanolamine (PE) are altered (18, 42). The ratio of PG/PE synthesis in uninfected cells is 0.35, whereas that of infected cells is 0.90. Chloramphenicol reduces the rate of phospholipid synthesis in uninfected cells to the rate found in phage-infected cells, but infection of chloramphenicol-pretreated cells reduces the synthetic rate still further. The change of the PG/PE ratio does not occur in the chloramphenicol-pretreated infected cells. Whether the change in rates of PG/PE synthesis is a primary effect of the phage is not entirely clear, as PG present in the cell prior to infection decays at twice the rate as in uninfected cells. The ratio of PG/PE synthesis also changes in stationary phase cells to 0.90 (42).

A WORD ABOUT BACTERIOCINS

Bacteriocins are a group of bacteriocidal, proteinaceous substances which are synthesized by certain strains of bacteria and act, usually from the cell surface, to inhibit the growth of the same or related species. Their genetic deter-

minants exist extrachromosomally in the cell, and are thus analogous to the determinants of some temperate phage. Some bacteriocins are small proteins which cannot be observed by electron microscopy, but others are seen to have structures very similar to phage although they do not contain DNA (15). Their action is therefore often compared to the action of phage ghosts. Several reviews concerning bacteriocins have been written (15, 37, 91, 101).

Colicins are a class of bacteriocins synthesized by certain strains of *Enterobacteriaceae* and act primarily on *E. coli*. The mode of action of colicins has been studied by Nomura (90), and it has been concluded that although they all do not have the same specific effects on the cell they all are acting through the cell membrane (80, 90). Evidence in support of this is the finding that trypsin can reverse the effect of colicins and that colicin-tolerant mutants can be isolated which have defective cell membranes (88, 90, 95). Changeux and Thiéry (20) proposed a specific model to explain how colicins could exert their various effects by acting through the membrane. The model is analogous to models for the allosteric regulation of some enzymes and is very similar to the model which we believe best explains the facts regarding early events in phage and ghost infection.

SUMMARY AND CONCLUSIONS

The following observations have been made regarding the inhibition of host functions after ghost infection. The attachment of one ghost to almost all cells which are susceptible to them causes a very rapid inhibition of the synthesis of all macromolecules, either of host or superinfecting phage origin, while leaving the energy-metabolizing systems of the host at least partially functional (29, 52, 76). A minor proportion of the cell population, whose exact number depends on the condition of the culture prior to infection, reacts variably to ghost infection—they synthesize some protein or some RNA, or both, but do not synthesize induced enzymes or support the multiplication of superinfecting phage. These cells later resume the production of host or superinfecting phage macromolecules and appear as colonies on overnight plates (30, 40). The proportion of these recovering cells is so low as to be undetectable if the cells have been grown in nutrient broth; if the cells are grown in synthetic media, 10% or more of the cells can recover. Several transport systems are no longer functional in ghost-infected cells (29), and the cell becomes permeable to several species of molecules (mainly flavin-related compounds) which could not enter the cell prior to infection (102, 114); there is a rapid loss of soluble nucleotides from ghost-infected cells (29, 52, 76, 98). Protein synthesis after ghost infection is not necessary for the ghost-induced inhibition to take place (29).

During the first several minutes of infection, phage-infected cells are not as drastically affected as are ghost-infected cells. In phage-infected cells, host protein and RNA synthesis may continue at a reduced rate for 3 min or more after infection (49, 62, 66, 71), although DNA synthesis and messenger RNA synthesis or translation, or both, are quite rapidly inhibited even in the absence of phage protein synthesis (7, 19, 22, 23, 49, 64, 93, 94, 96, 97, 109, 123, 126). Complete inhibition of all host macromolecular syntheses in phage-infected cells is seen only in the presence of protein synthesis, however (7, 33, 49, 64, 93, 96, 97). Some metabolites are observed to leak out of phage-infected cells for varying times post-infection (12, 29, 34, 98–100, 112, 116), although the absolute amount of loss from phage-infected cells is much less than from ghost-infected cells and may differ qualitatively (29, 98). Phage-infected cells are not inhibited in their ability to transport nucleic acid precursors even in the absence of protein synthesis (29). There are some membrane-associated phenomena that occur in phage infection but not in ghost infection—these include a refractoriness to lysis from without (125), the breakdown of superinfecting phage DNA (38, 39, 45), a delay in the time of lysis of some phage-infected cells by superinfecting phage (27), and changes in phospholipid metabolism (18, 42) and some transport systems (114, 115). The nucleotide pools in phage-infected cells are stabilized for several minutes postinfection and this, in the absence of nucleic acid synthesis, was interpreted to mean that the membrane has retained its normal function as a permeability barrier (29).

One hypothesis which is often called upon to explain the events which occur early after phage and ghost infection is the "hole puncture-repair" hypothesis (24, 99, 100, 116). This hypothesis assumes that a hole is made in the cell membrane during injection of the phage DNA and that this hole is later sealed by either host enzymes (116) or phage-induced enzymes (18, 42). In ghost-infected cells, the much greater leakage of metabolites and eventual death of the cell are hypothesized to be due to the lack of a repair mechanism to heal the cellular membrane (81, 122).

The concept of a membrane hole may have arisen originally for want of a more accurate description; it was perhaps not intended to be a literal description of what is actually taking

place, or it may have arisen due to some confusion as to the differentiation of function of cell wall and cell membrane. The concept, however, has been quite literally integrated into the body of knowledge concerning phage infection. The apparent swelling of cells after infection and the "leakiness" of infected cells do indeed support the concept of a hole. Membrane holes, however, are not a common occurrence. Although under some conditions cells can acquire a general increase in permeability, this cannot be attributed to true holes in the membrane because the permeability increase is not seen at 0 C and because the loss of metabolites from the cell is apparently not extensive—the cells grow at an undiminished rate (78). Nor is it necessary to postulate a hole for the entrance of the phage DNA. Transforming DNA enters the cell in the absence of hole puncture, as does isolated phage DNA, provided it is added to spheroplasts at the same time as urea-treated phage or ghosts (83, 86, 129). Because urea treatment causes the phage tails to contract (86), they cannot be postulated to be puncturing the membrane by the usually suggested method. Some restriction enzymes may act to break down DNA before it enters the cell, suggesting again that the phage tail does not penetrate the membrane (41).

Other facts argue against holes being instrumental after ghost infection. The oxygen uptake and respiratory quotient are unaffected in ghost-treated cells (40, 76), and these processes depend on critical concentrations of metabolites within the cell. Inorganic phosphate is assimilated for some time after ghost infection (76) and this process also depends on internal pools of metabolites.

When one compares the effects of phage and ghost infection, the results are even more difficult to reconcile with the concept of hole puncture. Presumably, phage-infected cells would leak metabolites until some repair process took place. The leakages from phage- and ghost-infected cells should therefore parallel one another until the leak is repaired. If, however, one looks at the soluble pools of nucleotides in the infected cells, it is seen that the losses from these pools after phage and ghost infection at no time parallel each other (29).

Several investigators (18, 42, 116) have presented evidence which supports the concept of repair. Silver et al. (116) observed that the K^+ leakage from phage-infected cells ceases within several minutes of phage infection, although protein synthesis is not necessary for this to take place, and several workers (18, 42) observed that phospholipid metabolism is altered after infection. Although these changes do undoubtedly take place, there is no evidence that they are part of a repair mechanism. Many changes which may be membrane associated take place after phage infection but, as they are initiated at and continue for various times postinfection, it is likely that a continuum of events is taking place on the membrane.

The hypothesis that I believe best fits all the known facts regarding the earliest events in phage and ghost infection is the following: that attachment of the phage protein coat to the cell wall causes events which result in allosteric changes in some membrane components and lead to functional detachment of the host DNA and protein-synthesizing systems, and also cause loss of other membrane-associated functions; the injection of the phage DNA or internal protein (or both) and its attachment to the cell membrane allows the membrane to retain its functionality, but in a slightly altered state that allows the production of phage macromolecules in lieu of host DNA and RNA (29).

If it is further supposed that after infection under some conditions the host chromosome can re-establish functional contact with the cell membrane, the findings of Landy and Spiegelman (71) and Kennell (66) that from 13 to 50% of the RNA synthesized after phage infection is host RNA and the observations of French and Siminovitch (40) on the recovery of ghost-infected cells could be explained. Why, teleologically, the phage needs a second mechanism to cause complete inhibition of host synthesis would also be explained. One can also imagine conditions under which the phage DNA is sufficiently damaged so as to be unable to cause the total inhibition of host syntheses (for which phage protein synthesis is necessary), but under which the DNA or internal protein, or both, of the phage would operate to allow the cell membrane to retain its functionality and thereby allow continued synthesis of host molecules. The situation just described may prevail in cases in which cells are infected with T* phage or with ultraviolet-irradiated phage (67, 118). The fact that some abortive infections are lethal and others are not could also be explained in this way. Smith et al. (117) have, in fact, shown that some strains of *E. coli* W have a greater ability to survive abortive infection by phage T2 if they can damage the phage DNA.

The hypothesis that some change in the membrane is responsible for the observed effects of phage and ghosts originated from the replicon hypothesis of Jacob and Brenner (63) which states that the chromosome is at some point in the cell cycle attached to the cell membrane, with the membrane acting to regulate replication and

division. That DNA is actually membrane bound has been shown in a variety of cases (103, 124), including T-even phage-infected cells (31); ribosomes have also been shown to be associated with the membrane (1, 107).

As previously mentioned, theories very similar to the one proposed here have been suggested for the mode of action of colicins (20, 80, 90), and several workers have supposed that the injected substance of the phage can in some way counteract the effects of the ghost (80, 112). Wyman (135) and Changeux et al. (21) showed that allosteric effects in extended systems are theoretically possible, and the paper by Changeux and Thiéry (20) pictorally illustrates how these could come about. Cohen (24) recently proposed a hypothesis for the inhibition of host functions after phage infection which is similar in some respects to these allosteric models, except that his model relies on rapid osmotic equilibrium caused by hole puncture to separate the bacterial genome and its transcribing apparatus. If one were willing to define increased permeability as "holes," the Cohen hypothesis would not be very unlike the allosteric hypothesis.

Perhaps the concept of allosteric changes in the membrane is only slightly more sophisticated than the concept of a hole, with both concepts merely serving to indicate that we do not know exactly what is going on. There is, in fact, no direct evidence that colicins, phage, or ghosts cause changes in the membrane, although in the case of colicins some tolerant mutants have been isolated and shown to have altered cell membranes (88, 90, 95). If ghosts are acting in a manner analogous to the way in which colicins act, it should be possible to isolate ghost-tolerant mutants which have altered cell membranes.

Although hole puncture and repair can, indeed, explain many of the events which occur after phage and ghost infection, it is my feeling that a model employing allosteric changes is more satisfactory than models based upon hole puncture, because the former can explain all the facts that can be explained by assuming that a hole has been made, and it can explain other observations as well. The allosteric model allows for specific changes in the permeability properties of both phage- and ghost-infected cells and it also explains how membrane function can be restored after phage infection but not after ghost infection, even in the absence of protein synthesis. Furthermore, specific predictions can be made on the basis of the allosteric model and it is hoped that in the testing of these predictions facts will come to light which will eventually lead to a clearer picture of exactly what events are taking place.

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